Catecholamine production in patients with gastroenteropancreatic neuroendocrine tumors

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Abstract

Objective: Amine precursor uptake and decarboxylation is a classical feature of gastroenteropancreatic (GEP) neuroendocrine tumors (NET). Production of catecholamines was studied in GEP NET and non-NET patients.

Design: A cross-sectional study was undertaken.

Methods: We studied catecholamine and metabolite secretion in 115 consecutive GEP NET patients and in 20 patients with non-NET. After specific extraction, vanilmandelic acid, homovanilic acid, catecholamines (norepinephrine, epinephrine, dopamine) and methoxylated derivatives (metanephrine, normetanephrine, methoxytyramine) in urinary extracts were analyzed by high performance liquid chromatography. Results were indexed to the 24-h urinary creatinine levels.

Results: Among the 115 patients with NET, 9 (8%) had an increase of at least one urinary catecholamine or metabolite; in 7 out of the 9 the increase was slight being less than twice the upper value of the normal range. Elevated urinary dopamine (3 patients), methoxytyramine (6 patients), norepinephrine (2 patients) and normetanephrine (2 patients) were found. No increased urinary excretion of epinephrine nor metanephrine was observed. An adrenal mass existed in one of these nine patients but metaiodobenzylguanidine scintigraphy was negative as was immunohistochemistry for epithelial markers. None of the 20 patients with non-NET demonstrated an increased excretion of catecholamine or metabolites. No relationships were found between catecholamine and metabolite excretions and patients' tumor and treatment characteristics.

Conclusion: Production of catecholamines and metabolites is a rare event in GEP NET patients. Histological results, including positive immunohistochemistry for epithelial markers may help to diagnose GEP NET.

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Introduction

Gastroenteropancreatic neuroendocrine tumors (GEP NET) are classified according to their embryological origin as foregut- (respiratory tract, stomach, duodenum and pancreas), midgut- (ileum and right colon) or hindgut (left colon and rectum)-derived NET. They are embryologically of endodermal origin. GEP NET can produce multiple hormones including various peptides and glycoprotein hormones (1). They are also capable of amine precursor uptake and decarboxylation (APUD) leading to biogenic amine secretion (2). Because of these biochemical properties, Pearse initially named neuroendocrine tumors 'APUDomas' (3). These hormonal secretions participate in the clinical presentation and prognosis of GEP NET. Production of serotonin and histamine is known to mediate part of the carcinoid syndrome observed in midgut and foregut NET (4). In contrast, catecholamine production in GEP NET has rarely been studied (5–9). Nevertheless, GEP NET

tissues are known to contain substantial amounts of catecholamines (5–7, 9), to express synthesizing enzymes of the catecholamine metabolite pathway (7), and a role for catecholamine secretion has been suggested in the pathogenesis of the carcinoid flush (10–12). However, the therapeutic impact of catecholamine secretion on carcinoid syndrome management remains debatable.

Using specific methods, urinary excretion of catecholamines and derivatives was studied in 115 consecutive patients with GEP NET followed in a single center.

Patients and methods

Patients

Between 1993 and 1996, 115 consecutive patients with a histologically confirmed GEP NET were included in this study. There were 61 males and 54 females, with

a mean age of 54 years (range: 27–76 years). Among them, 73, 26 and 4 patients had a foregut-, a midgut-, or a hindgut-derived tumor respectively, and 12 patients had a NET of unknown primary site. All tumors disclosed NET morphological features including regular cells, normochromatic nucleus, and eosinophilic cytoplasm arranged in ribbons, nests or sheets separated by a fine fibrovascular stroma. An immunohistochemical study with neuron-specific enolase, chromogranin A and synaptophysin antibodies (DAKO, Glostrup, Denmark) was performed when the morphological structure precluded an unequivocal diagnosis of NET. Immunohistochemistry for an epithelial marker (cytokeratin (CK 22), DAKO) was used to exclude a pheochromocytoma when needed since GEP NET are positive for the epithelial marker unlike pheochromocytomas (13). At the time of the study all patients had active disease: 110 (95%) had distant metastases and 5 had a locoregional disease. Ninety-five patients had already been treated with surgery, chemotherapy, somatostatin analogs, interferon or external radiotherapy. The mean follow-up time from initial therapy was 53 months (range, 2–306 months). Twenty patients who were treated with chemotherapy for a non-NET were studied as controls of the stress induced by cancer. This group consisted of 9 males and 11 females, with a mean age of 56 years (range: 37–76 years). All had distant metastases from an anaplastic thyroid carcinoma ($n = 3$), a papillary thyroid carcinoma ($n = 3$), a lymphoma ($n = 5$), a breast carcinoma ($n = 2$), a colon carcinoma ($n = 2$), or various primary sites ($n = 5$).

Methods

Twenty-four hour urine samples to be tested for catecholamines and their metabolites were collected into chemically clean polyethylene vessels containing 15 ml 6 mol/l hydrochloric acid. The collection was done before chemotherapy and without any dietary restriction. Analytical procedures were performed as previously described (14, 15). In order to increase both the sensitivity and the specificity of these measurements, all results were indexed on 24-h urinary creatinine. Vanilmandelic acid (VMA, normal value $<6 \mu\text{mol}/\text{mmol}$ creatinine), homovanilic acid (HVA, normal value $<10 \mu\text{mol}/\text{mmol}$ creatinine) and 5-hydroxyindolacetic acid (5-HIAA, normal value $<10 \mu\text{mol}/\text{mmol}$ creatinine) were extracted from acidified urine using a VMA/HVA/5-HIAA reagent kit (Bio-Rad Laboratories, Munchen, Germany). Free catecholamines (norepinephrine, normal value $<60 \text{ nmol}/\text{mmol}$ creatinine; epinephrine, normal value $<15 \text{ nmol}/\text{mmol}$ creatinine; dopamine, normal value $<400 \text{ nmol}/\text{mmol}$ creatinine) were extracted by using a cationic resin followed by isolation on alumina. Total methoxylated derivatives (metanephrine, normal value $<400 \text{ nmol}/\text{mmol}$ creatinine; normetanephrine, normal value $<500 \text{ nmol}/\text{mmol}$ creatinine; methoxytyramine, normal value $<350 \text{ nmol}/\text{mmol}$

creatinine) were extracted by using a weakly acidic cationic resin and then a strongly basic anionic resin (Bio-Rad Laboratories). Standard control urine samples were extracted in the same run as patient urine samples and an appropriate internal standard was added to all samples before extraction. All extracts were then analyzed by high performance liquid chromatography with a reverse phase column and an amperometric detection. This study was performed after obtaining informed consent from the patients.

Relationships between catecholamine and metabolite secretions and demographic characteristics (age, sex), tumor features (embryological origin, disease extent, 5-HIAA secretion) and previous therapies were sought. Fisher's exact test was used to compare proportions, whereas means were compared using non-parametric tests: Wilcoxon's test or Kruskal-Wallis' test when there were more than two means.

Results

Among the 115 patients with NET, 9 (8%) had an increased excretion of at least one urinary catecholamine or metabolite (Table 1). In 7 patients, the increase was slight, being less than twice the upper value of the normal range. Three patients (3%) had an increased excretion of dopamine, 6 (5%) of methoxytyramine, 2 (2%) of norepinephrine and 2 (2%) of normetanephrine. Among the 9 patients increased excretion of dopamine and methoxytyramine was found in two, of methoxytyramine and norepinephrine in two and of dopamine, methoxytyramine and norepinephrine in one patient. No increased urinary excretion of epinephrine or metanephrine was observed. In these nine patients, CT scans and ultrasonography of the adrenals were normal in eight and revealed an adrenal mass in one, the evolution of which paralleled other metastases; metaiodobenzylguanidine (MIBG) scintigraphy performed in four patients was negative in three, including the patient with an adrenal mass, and demonstrated only a low hepatic metastatic uptake in one. Immunohistochemistry for an epithelial marker was negative in the patient with the adrenal tumor. Among the 9 patients, 8 had a foregut-derived tumor, including 4 pancreatic and 4 respiratory tract tumors. One patient had a midgut-derived tumor. An increased urinary excretion of 5-HIAA was observed in 51 of the 115 patients (44%). Among the 9 patients with an increased excretion of catecholamines or derivatives, excretion of 5-HIAA was increased in 4 and normal in the other 5 patients. No personal and/or familial history of NET was found in any of these 9 patients.

No increased urinary excretion of catecholamines and derivatives and of 5-HIAA was observed among the 20 control patients.

No relationships were found between catecholamine and metabolite secretions and patients' tumor and treatment characteristics.

Table 1 Patients' characteristics.

Age (years)/ sex	Primary site	Metastases	Carcinoid syndrome	MIBG ¹	Adrenal CT ¹	5-HIAA* (μ mol/mmol Cr)	NE* (nmol/mmol Cr)	NM* (nmol/mmol Cr)	DA* (nmol/mmol Cr)	MT* (nmol/mmol Cr)
54/F	Pancreas	Yes	Yes	Neg	Neg	—	—	—	627	—
44/F	Pancreas	Yes	No	ND	Neg	—	—	544	—	—
68/M	Lung	Yes	Yes	ND	Neg	120	—	544	—	—
68/F	Lung	Yes	Yes	ND	Pos	—	—	—	—	580
48/M	Ileum	Yes	Yes	ND	Neg	179	126	—	—	749
39/F	Pancreas	Yes	Yes	ND	Neg	22	—	—	—	826
54/M	Lung	Yes	No	Neg	Neg	—	—	—	—	916
56/F	Pancreas	Yes	No	Neg	Neg	—	—	—	693	1900
45/M	Lung	Yes	No	Pos (liver)	Neg	39	78	—	5365	3942

* 5-HIAA, normal levels < 10 μ mol/mmol creatinine; NE: norepinephrine, normal levels < 60 nmol/mmol creatinine; NM: normetanephrine, normal levels < 500 nmol/mmol creatinine; DA: dopamine, normal levels < 400 nmol/mmol creatinine; MT: methoxytyramine, normal levels < 350 nmol/mmol creatinine. Only abnormal values of biogenic amines are given. Cr: Creatinine; ¹Pos: positive; Neg: negative; ND, not done.

Discussion

This study shows that using specific methods, 8% of patients with metastatic gastroenteropancreatic neuroendocrine tumors have an increased urinary excretion of catecholamines or derivatives. Most patients had advanced disease and recommendations could not be applied directly to patients with limited disease.

In most patients, this increase was slight and was much lower than that observed in the majority of patients with pheochromocytomas, especially when malignant (16). It was fivefold higher than the upper level of the normal range in only two patients. An adrenal mass existed in one of these nine patients, the evolution of which paralleled that of other metastases and metaiodobenzylguanidine scintigraphy was weakly positive demonstrating an hepatic focus of low uptake in only one patient. Differential diagnosis between GEP NET and pheochromocytomas may arise, either in patients presenting with a metastatic GEP NET with adrenal tumors or between a GEP NET and an ectopic pheochromocytoma. Since on the one hand, catecholamine production, as well as MIBG uptake can be found in GEP NET and on the other hand, a negative MIBG scintigraphy and absence of catecholamine production can be observed in pheochromocytoma especially when ectopic, we suggest that in these patients, immunohistochemical results with negative epithelial markers may be the main tool for the diagnosis of GEP NET (13); in fact, this was found in our patient with increased production of catecholamine and an adrenal tumor.

In the absence of urinary excretion of epinephrine and of its metabolites, production of dopamine and norepinephrine metabolites may be related either to catecholamine secretion by adrenergic nerve endings, which can be induced by serotonin (17), or to a direct release from the primary GEP NET. Since, the catecholamine production was not related to that of serotonin, as indicated by urinary 5-HIAA excretion, the first hypothesis is unlikely. Similarly, stress, previously suggested as the cause of this overproduction (8, 9) can be excluded since no control patient had an increased excretion of catecholamines and metabolites. Normalizing 24-h urinary catecholamine levels to creatinine excretion may help to decrease the number of false positive results (18). Hence, cancer should not be considered as associated with increased neuronal catecholamine secretion. Therefore, this production is likely to arise from the NET itself. Indeed, enzymes of the catecholamine-synthesizing pathway, such as tyrosine hydroxylase, aromatic L-amino acid decarboxylase and dopamine β -hydroxylase are expressed in these tumors, suggesting that a synthesis of catecholamines may occur in these tumors (7). Catecholamine excretion concerns only dopamine, noradrenaline and derivatives, demonstrating that this synthesis of catecholamines could be partly defective in these tumors.

Our results differ from those of two previous large series on 64 and 44 GEP NET patients (8, 9), both employing sensitive and specific methods for catecholamine and metabolite measurements. Both series reported elevated urinary dopamine and/or metabolites in 12–38% of patients and elevated urinary norepinephrine and/or metabolites in 18–33% of patients, depending on the embryological origin (9) or presence or absence of serotonin production (8). We found a lower frequency of catecholamine and metabolite secretion, which may be explained by the higher number of patients studied, fluctuating rates of catecholamine secretion as previously reported (8, 9), and indexation of catecholamine and metabolite secretion to creatinine levels. Also, only one measurement of catecholamines and metabolites was performed for each patient in our study instead of two or more in a previous study (9). Furthermore, no increased production of epinephrine or of its metabolites was found in our study, in contrast with some previous results (5, 6, 9). Nevertheless, since the presence of phenylethanolamine-N-methyltransferase has not been demonstrated in GEP NET, it remains unclear whether epinephrine excretion reported by one previous study is also caused by GEP NET. Finally, most patients with elevated catecholamine and metabolite excretion had a foregut-derived tumor. This is in sharp contrast with a previous study (7) in which most patients with catecholamine overproduction had a midgut-derived tumor. Clinical presentation, histological pattern and previous therapy of GEP NET patients may have influenced these results.

In conclusion, increased production of catecholamines was found to be a rare event in GEP NET patients. Predominant secretions are dopamine, norepinephrine and metabolites, with only slight increases in most cases. Histological results, including immunohistochemistry for epithelial markers may help to differentiate GEP NET from pheochromocytomas.

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